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Best Practices for the Use of Micropipets

Every day, air-displacement pipets are used to quantitatively dispense sample and reagent aliquots for reaction, routine analyses, and specialized tests. Since concentrations of biological and chemical components in the prepared samples for analyses and assays are volume-dependent, incorrectly performed pipetting steps will directly impact the transferred volumes, and hence, the test results. The design and construction of piston-operated air-displacement pipets render their performance susceptible to the pipetting technique and skills used by the operator of such devices. The pipet operator usually has the ability to mitigate the influence of most parameters by using the appropriate technique, as well as by choosing the appropriate pipet size and type of pipet tips.

Influence of the air cushion on pipet performance

Piston-operated air-displacement pipets use an air cushion to couple the pipet's piston to the aspirated liquid inside of the pipet tip. This air cushion, often referred to as captive air volume or dead air volume, is trapped within the pipet as soon as the tip is immersed in the sample solution. This captive air volume closely obeys the Ideal Gas Law (P_a is the pressure of the trapped gas, V_a its volume, n_a the number of moles, and T_a the temperature of the gas):

$$P_a V_a = n_a R T_a$$

The Ideal Gas Law allows one to estimate the effects that temperature and, by extension, evaporation, and the ratio of captive air volume to the pipet's set volume will have on the actually aspirated and delivered volume of a pipetting cycle.[1] The following techniques studied here directly influence the captive air volume: prewetting of pipet tips, temperature disequilibrium, hand warming, and immersion depth of pipet tip.

Since the total volume of the air cushion can vary widely depending on the type of pipet, the tip type and size, and the amount of the aspirated liquid aliquot, this study evaluated two different scenarios: one set of experiments was conducted with a 20-µL pipet set at 20 µL, and the other experiments with a 100-µL pipet set at 20 µL.

Prewetting of pipet tips

Sample solution in the pipet tip is susceptible to evaporation into the air cushion during and after aspiration. The evaporative loss of sample solution is dependent on the humidity of the captive air space, as well as the temperature of the sample solution. Repeated aspiration/dispense cycles will increase the humidity of the air in the pipet tip and shaft. *Figure 1* shows the dispensed volumes of a 20-µL pipet set to 20 µL and used in a noncontrolled laboratory (30% relative humidity). Each dispense was performed with a new tip. Without prewetting the tips, the pipet dispensed on average 1.3% less volume, as compared to dispenses when the tip was prewetted three times prior to the dispense. When using pipets in particularly dry or warm environments, the error can be significantly larger without prewetting the tips.[2]

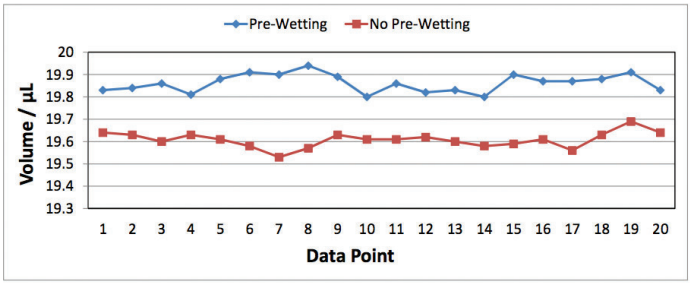

Figure 1 – Volume deliveries of a 20-µL pipet. Each delivery used a new tip and was either prewet three times or not prewet prior to sample delivery.

Temperature disequilibrium

For most accurate pipetting results, it is recommended that the pipet, the pipet tip, and the sample solution have been equilibrated for at least 2 hr and are within 0.5 °C of ambient temperature.[3] Many samples, however, must be handled at specific high or low temperatures, and pipetting such samples can introduce significant errors in the delivered volume due to the expansion or contraction of the captive air volume and evaporation. Studies of this effect have been reported previously.[4]

The present study (see experimental conditions) evaluated the use of pipet tips that had been cooled to 4 °C for 30 min prior to use. Pipetting with these cold tips led to significant underdelivery of sample with both pipets, contributing up to −1.9% relative inaccuracy (RI) and 1.2% coefficient of variance (CV) to the errors. The inaccuracy and imprecision results for both pipets and all tested scenarios described here are graphed in *Figures 2–5*. Experimental conditions are shown in *Table 1*.

Heat transfer/hand warming

Handling a pipet for prolonged periods of time will cause the barrel of the pipet to warm, leading to an expansion of the captive air volume, ultimately impacting the accuracy and precision. Progressive warming of the pipet's barrel through heat transfer from the hand manifests itself by a trend toward smaller delivered volumes, and led to −1.1% RI and 0.8% CV in this study.

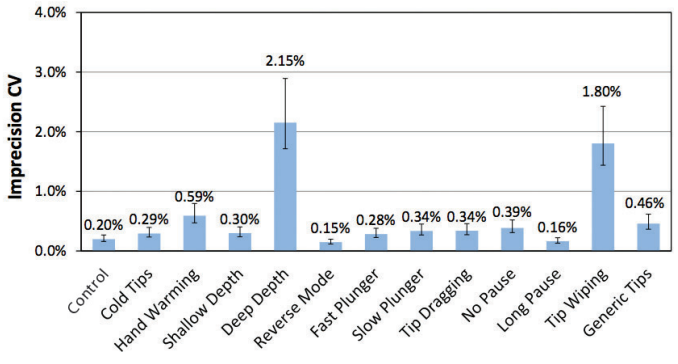

Figure 2 – Imprecision (CV) of a 20-µL pipet set to 20 µL.

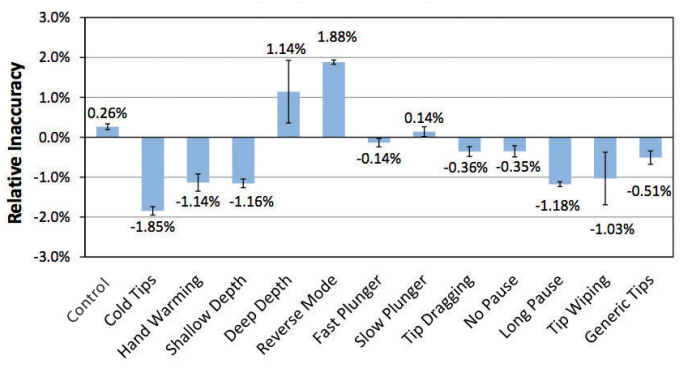

Figure 3 – Inaccuracy of a 20-µL pipet set to 20 µL.

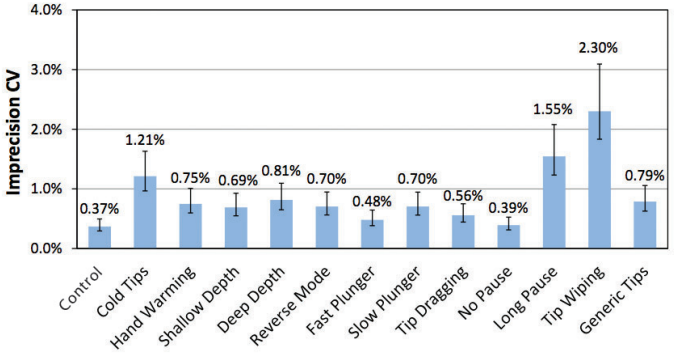

Figure 4 – Imprecision (CV) of a 100-µL pipet set to 20 µL.

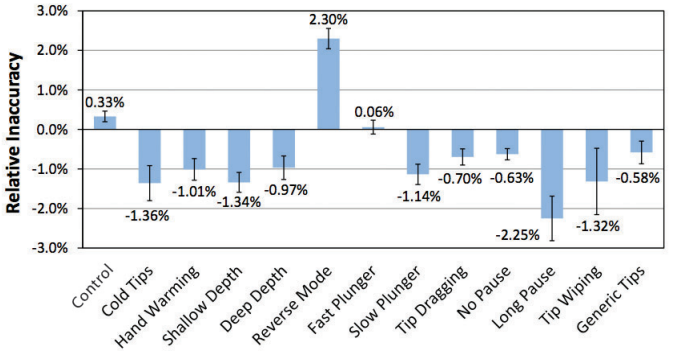

Figure 5 – Inaccuracy of a 100-µL pipet set to 20 µL.

Immersion depth of the pipet tip

Immersing the pipet tip to the proper depth during aspiration of the sample is important. Pipet calibration standards like ASTM E1154 recommend[5] an immersion depth of 2–3 mm for pipet volumes of 1–100 µL, 2–4 mm for 101–1000 µL, and 3–6 mm for volumes larger than 1 mL. In this study we evaluated immersion depths of 1 mm and 8 mm. A shallow immersion depth increases the risk of aspirating small amounts of air, while immersing tips too deeply increases the risk of carrying over droplets on the outside of the tip, and/or forcing more sample in the tip due to increased hydrostatic pressure on the outside of the tip. Either case leads to a significantly increased imprecision (up to 2.2% CV) of the delivered volumes.

Table 1 – Experimental conditions

- All tests reported in Figures 2–5 were conducted in a controlled calibration laboratory, at 20.0 ± 1.0 °C and 45–65% relative humidity.
- Volume measurements were performed with an **Artel** PCS® Pipette Calibration System (Westbrook, ME), using the photometric method according to ISO 8655-7.
- Only one parameter of the pipetting technique was varied in each experiment and compared to the control method described below; compounding of technique errors was not investigated in this study.
- Two pipets were evaluated in this study:
  - 1) 20-µL pipet set at 20 µL
  - 2) 100-µL pipet set at 20 µL
- The manufacturer's recommended tips were used.
- Experiments were carried out by trained operators.
- The control method used the following pipetting technique:
  - Each pipet tip was prewet 3 times
  - The tip was immersed 2 mm below the meniscus in the sample solution
  - Pipet was held in a vertical position during aspiration, and at a 45° angle during the dispense against the glass wall of the measurement cuvette
  - Forward mode of pipetting was used, with blow-out during dispensing
  - Each experiment was conducted with 30 replicates
  - A new pipet tip was used for each data point.
- Accuracy is reported as relative inaccuracy (RI) as percent difference to the set volume of the pipet.
- Precision is reported as the coefficient of variance (CV).

Forward and reverse mode

Use of the appropriate pipetting mode has one of the biggest influences on the accuracy of the volume delivery. In forward mode, the plunger is depressed to the first stop, the pipet tip is then immersed in the sample solution, and the sample is subsequently aspirated. During delivery, the plunger is depressed beyond the first stop (blow-out stop), forcing all the liquid out of the tip. Standard procedure for pipet calibration prescribes using this forward mode and aqueous sample solutions.[3,5]

In reverse mode, the plunger is depressed beyond the first stop (to the second stop) before immersing the tip in the sample, aspirating more

than the desired sample volume. The desired volume is delivered by depressing the plunger to the first stop, retaining the additional sample in the tip. While this pipetting mode is recommended for use with viscous or volatile solutions, using reverse mode with aqueous solutions leads to significant overdelivery of up to 2.3% RI and contributes up to 0.7% CV.

Consistent plunger speed and pressure

Depressing and releasing the plunger with consistent speed during aspiration and dispensing of the liquid aliquot is important for achieving precise and accurate results. The type of pipet, tip, and sample solution will determine the optimum pressure needed to move the plunger with a consistent and appropriate speed. Our studies indicate that a slow aspiration speed may result in underdelivery of up to −1.1% RI and contribute up to 0.7% CV.

Position of tip during aspirating and dispensing

Holding the pipet in a vertical orientation and preventing the tip from touching the side or bottom of the sample vessel will ensure an optimal and undisturbed hydrodynamic flow of the sample during aspiration. Further, it is important not to drag the tip along the wall of the source vessel after aspiration, because this may lead up to −0.7% RI and 0.6% CV.

When dispensing the sample, it is recommended to touch the pipet tip against the side of the receptacle, while the pipet may be held at a 45° angle. With the exception of pipetting very small volumes, it is not recommended to immerse the tip into already present solution in the receiving vessel, because this may lead to overdelivery if droplets are clinging to the outside of the tip, and significantly increases the risk of cross-contamination.

Pause after aspirating

Once the aliquot of sample solution has been aspirated into the pipet tip, it is important to pause for about 1 sec with the tip still immersed in the source liquid, allowing the sample to "settle" in the tip. Removing the pipet tip prior to allowing the vibrational motion of the liquid to settle will introduce errors in the precision and accuracy, up to −0.6% RI and 0.4% CV in our studies. Allowing the tip to remain in the liquid for too long, however, will result in significant underdelivery, up to −2.3% RI and 1.6% CV. The magnitude of these errors depends on the pipet tip, temperature, sample type (vapor pressure), speed of aspiration, and sample volume.

Tip wiping

The practice of wiping the pipet tip after aspiration with a laboratory cloth is widespread. Due to the high propensity of introducing large errors through this technique, one should be very carefully evaluate whether this step is really necessary. If it is determined that a particular sample is prone to forming droplets on the outside of the pipet tip that must be wiped off, extreme care should be taken not to touch the tip orifice, since it is very easy to wick out some of the sample solution. In our study, tip wiping introduced over 2.3% of CV and led to underdelivery of up to −1.3% RI.

Pipet tip quality

For the most accurate and precise pipetting results, the pipet manufacturer's recommended tips should be used. Achieving a proper seal between the pipet's nose cone and the tip is critical for good performance. Some generic tips may seemingly fit on a pipet, but due to different taper angles of the nose cone and tip, a poor seal is established, resulting in errors. In our study, the generic tips fit on the pipets but still introduced errors of up to −0.6% RI and 0.8% CV, which would be additive to all other pipetting errors. If high-quality third-party tips are to be used, it should be verified that they fit well and form a tight seal with the intended pipet model.

Claimed pipet performance assumes the use of manufacturer's tips. When calibrating a pipet, it is imperative that it be calibrated with the same tip type and under the same conditions of its use in the lab in order to avoid errors when using the pipet for analytical tests.

Pipet size

Adjustable-volume pipets can be used over a large range of volumes. Manually operated pipets usually allow the user to select volumes as low as 10% of the pipet's nominal volume, while some electronically operated pipets offer an even wider range of selectable volumes. Best pipet performance, however, is achieved at or near the nominal volume of a pipet. For best results, it is recommended to use variable-volume pipets only down to the nominal volume of the next available, smaller denomination of pipet.

Best pipetting practices

The results of this study demonstrate that even minor variation in the operating technique of handheld air-displacement pipets can result in measurable errors in accuracy and precision. This study did not evaluate errors resulting from combining multiple of the discussed technique variations, although this is commonly observed in the field. Compounded errors can easily reach 12%, and are often even larger, as data from field surveys suggest.

The following steps will ensure the most accurate and precise results:

- Prewet tips at least three times
- Use proper pipetting mode
- Work at temperature equilibrium
- Immerse tips to proper depth
- Aspirate with pipet in vertical position
- Pause after aspirating
- Do not touch vessel wall during or after aspiration
- Use consistent plunger speed and pressure
- Minimize heat transfer from hands
- Avoid tip wiping
- Examine tip prior to dispensing
- Use high-quality pipet tips
- Use proper pipet size.

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